

Towards Resolving the Presynaptic NMDA Receptor Debate

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Abstract

In the classical view, postsynaptic NMDA receptors (NMDARs) trigger Hebbian plasticity via Ca^{2+} influx. However, unconventional presynaptic NMDARs (preNMDARs) which regulate both long-term and short-term plasticity at several synapse types have also been found. A lack of sufficiently specific experimental manipulations and a poor understanding of how preNMDARs signal have contributed to long-standing controversy surrounding these receptors. Although several prior studies linked preNMDARs to neocortical timing-dependent long-term depression (tLTD), a recent study argues that the NMDARs are actually postsynaptic and signal metabotrophically, i.e. without Ca^{2+} . Other recent work indicates that, whereas ionotropic preNMDARs signaling controls evoked release, spontaneous release is regulated by metabotropic NMDAR signaling. We argue that elucidating unconventional NMDAR signaling modes — both presynaptically and metabotrophically — is key to resolving the preNMDAR debate.

Highlights (three to five, 85 characters each, with spaces)

- Controversial preNMDARs regulate long and short-term plasticity
- PreNMDARs may signal ionotropically, depending on Ca^{2+} and Mg^{2+}
- PreNMDARs may signal metabotropically, without Ca^{2+} or Mg^{2+} dependence
- Elucidating preNMDAR signaling paths will help settle controversy

Introduction

In the classical view, NMDARs act as postsynaptic coincidence detectors that trigger Hebbian plasticity via Ca^{2+} influx [1,2]. PreNMDARs have been relatively overlooked compared to their postsynaptic counterparts, yet have for decades been found at numerous synapse types, where they influence long- as well as short-term plasticity [3-5].

PreNMDARs have been the subject of considerable debate [3-5]. One reason for this is a lack of sufficiently specific experiments, which has raised several questions about preNMDARs: Where precisely at the synapse are they located, and how do they influence synaptic plasticity and transmitter release? Here, we discuss recent studies that address these questions.

PreNMDARs influence long-term plasticity

Like postsynaptic NMDARs [1,2], preNMDARs determine long-term synaptic plasticity [3,5,6]. However, typically only a subset of synaptic boutons express preNMDARs [7], and expression is determined by the postsynaptic target neuron's identity [6,8,9]. For example, synapses from neocortical layer 5 (L5) pyramidal cells (PyCs) to neighboring L5 PyCs express functional preNMDARs, but synapses from PyCs to neighboring basket cells do not [9]. Since preNMDARs are a key determinant of neocortical timing-dependent long-term depression (tLTD) [10], this expression pattern results in synapse-type-specific plasticity [4,11]. This heterogeneous expression may also explain why axonal preNMDAR Ca^{2+} signals have not always been found [12-15].

Another possible explanation for why preNMDARs have been difficult to detect experimentally is that preNMDARs may not always conduct Ca^{2+} like their postsynaptic counterparts. At connections between cortical layer 4 (L4) spiny stellate cells and layer 2/3 (L2/3) PyCs in somatosensory cortex (**Fig. 1**) and between CA3 and CA1 neurons in the hippocampus,

preNMDARs involved in tLTD contain the GluN2C/GluN2D subunits [16,17], which lower Mg^{2+} sensitivity and Ca^{2+} permeability [18]. Low preNMDAR Ca^{2+} conductance has also been suggested at visual cortex L4-L2/3 synapses [8,19], where tLTD depends on both GluN3A- and GluN2B-containing NMDARs, which confer low and high sensitivity to Mg^{2+} , respectively [18]. This form of tLTD may thus involve heterotrimeric preNMDARs that are less sensitive to Mg^{2+} and that flux less Ca^{2+} [18].

However, in disagreement with the prior literature [8,19-25], Carter and Jahr [12] recently proposed that preNMDARs are not required for tLTD at L4-L2/3 connections in somatosensory cortex. This finding resembles previously described results at L2/3-L2/3 synapses where, in contrast to L4-L2/3 connections, it is well established that tLTD relies on post- but not presynaptic NMDARs [16,21,26,27]. As Carter and Jahr [12] did not show that the Channelrhodopsin-2 expression was specific to L4, this study may lack specificity for presynaptic cell type, possibly explaining why their findings resemble the previously reported postsynaptic tLTD at L2/3-L2/3 synapses and not preNMDAR-mediated tLTD at L4-L2/3 connections [8,19-25]. Interestingly, Carter and Jahr [12] reported that the postsynaptic NMDARs involved in tLTD at this synapse signaled metabotroically, i.e. without the need for Ca^{2+} flux (**Fig. 1**). Although relatively overlooked, metabotropic NMDAR signaling [28,29] — which is mediated by conformational changes rather than ionic flux [30,31] — has interestingly been recently linked to classical hippocampal LTD [32], spine shrinkage [33], and Alzheimer's disease [32,34]. At some synapse, tLTD may thus involve postsynaptic NMDARs signaling metabotroically [12], but it remains unclear precisely which synapses.

Carter and Jahr's [12] study highlights the acute need for an improved understanding of unconventional NMDAR signaling modes [28], because there may be several ways of inducing

NMDAR-dependent plasticity. For example, although hippocampal CA1 plasticity has traditionally been viewed as postsynaptic [35-37], recent findings by Andrade-Talavera *et al.* [17] indicate a dissociation: Hippocampal CA1 tLTD depends on preNMDARs and timing-dependent long-term potentiation (tLTP) on postsynaptic NMDARs. A similar dissociation between tLTD and tLTP has previously been found at several neocortical synapses [10,23,24,38], hinting that this is a general principle. Additionally, Andrade-Talavera *et al.* [17] found that CA3-CA1 tLTD depended on non-postsynaptic ionotropic NMDAR signaling and on presynaptic calcineurin, which could be the target of preNMDAR-mediated Ca^{2+} (**Fig. 1**) [17]. Recently, Padamsey *et al.* [39] demonstrated that GluN1 deletion in CA3 abolished presynaptic LTD, strengthening the evidence for preNMDARs underpinning CA3-CA1 LTD.

PreNMDARs have also been found at several cerebellar synapse types [6]; we focus here on parallel fiber (PF) to Purkinje cell (PuC) synapses. As opposed to neocortical plasticity, PF-PuC plasticity follows non-Hebbian rules [40]: Pairing PF and PuC stimulation within a ± 100 ms temporal window elicits LTD. To induce LTP, only PF stimulation is required [41]. Similar to neocortical tLTD, PF-PuC LTD depends on preNMDARs. PF-PuC LTD is expressed postsynaptically via NMDAR-dependent anterograde diffusion of nitric oxide (NO) (**Fig. 2**) [6,42-44]. Recently, using GluN1 deletion in granule cells, Bouvier *et al.* [43] demonstrated that preNMDARs are essential for PF-PuC LTP. Two-photon Ca^{2+} imaging showed that only high-frequency activation elicited preNMDAR Ca^{2+} transients, in keeping with the frequency dependency of LTD and LTP [43,45]. PreNMDARs at PFs thus implement a high-pass filter for burst detection, a property presumably inherited from the voltage-dependent Mg^{2+} blockade of GluN2A-containing NMDARs [43,45]. Spread of dendritic NMDAR signals in presynaptic cells [14,15] cannot account for these results, since boutons were severed from somata [as in 46].

Using 2-photon imaging, Bouvier *et al.* [43] only found preNMDARs in a subset of PF boutons. This heterogeneity of preNMDAR expression is reminiscent of that reported in visual cortex [8,9]. In the latter case, however, preNMDAR expression is specific for synapse type. Nevertheless, heterogeneity may be a general principle governing preNMDARs [4], which sets them apart from postsynaptic NMDARs [9,47].

PreNMDARs also influence short-term plasticity

In addition to LTP and LTD, preNMDARs regulate short-term plasticity (STP) [10,47], encompassing both facilitation and depression, with a time course ranging from milliseconds to minutes [48]. STP adds diversity of signaling in local circuits, because synapses from the same neuron onto different target neuron types can express widely different forms of STP [9]. This means STP is synapse type specific [11], just like long-term plasticity [4].

Since preNMDARs are typically sensitive to Mg^{2+} [3,5], at least two action potentials (APs) in rapid succession should be required to activate preNMDARs, at least if they operate as autoreceptors [although see 49,50]. This is because the AP is so brief that when glutamate binds preNMDARs, they remain Mg^{2+} blocked unless depolarized by subsequent APs. In agreement, such frequency-dependent activation of preNMDARs has been reported in visual cortex [8,10,47], hippocampus [51] and cerebellum [43,45]. Mg^{2+} -sensitive preNMDARs can thus be thought of as detectors of high-frequency presynaptic firing. In neocortex, the critical frequency for preNMDAR activation is ~ 8 Hz [47] (although hippocampal preNMDARs bandpass filter around ~ 5 Hz [51]).

One apparent caveat with this interpretation is that preNMDARs apparently modify release probability (p_r) at the first AP in a high-frequency train [8-10,21,47], when preNMDARs should presumably still be Mg^{2+} blocked. Recently, Abrahamsson *et al.* [47] proposed a solution to this

conundrum: PreNMDARs may regulate p_r indirectly, by modifying the replenishment rate of the readily releasable pool (RRP) (**Fig. 3**), which would affect p_r at all APs in a high-frequency train, including the first. This interpretation may hold true for preNMDARs with frequency-dependent regulation of STP, such as those at L4-L2/3 synapses [8,21] or at hippocampal CA3-CA1 synapses [51]. As a general principle, preNMDARs may thus serve to upregulate RRP replenishment rates during periods of elevated activity, to sustain p_r .

Regulation of spontaneous release by preNMDARs

Neocortical preNMDARs generally modulate both evoked and spontaneous release [8-10,19,47]. This may seem peculiar, since preNMDAR regulation of evoked release requires presynaptic spiking above ~8 Hz [47], but spontaneous release rates are typically considerably lower [9,10]. Yet, the preNMDARs that regulate evoked and spontaneous release both contain the GluN2B subunit [10], implying that preNMDAR regulation of spontaneous release should also be Mg^{2+} sensitive [18] [but see 19]. This apparent discrepancy has been a long-standing issue in the field [3,5,10,21].

Although evoked and spontaneous release were initially thought to rely on the same molecular machinery, the emerging consensus is that the two forms of release employ distinct pathways [52]. In agreement, Abrahamsson *et al.* [47] found that preNMDARs at excitatory synapses onto visual cortex L5 PyCs regulate evoked and spontaneous release via non-overlapping pathways (**Fig. 3**). PreNMDARs rely on the active-zone protein RIM1 $\alpha\beta$ [53] to regulate evoked but not spontaneous release. Conversely, preNMDARs require JNK2 [54] to control spontaneous release — as previously shown in entorhinal cortex [55] — but not evoked release. These findings thus revealed a double dissociation of preNMDAR signaling in controlling transmitter release.

Still, the absence of frequency dependence in preNMDAR regulation of spontaneous release was enigmatic. An explanation might be found in metabotropic NMDAR signaling, since it is not Mg^{2+} dependent [32]. Consistent with metabotropic signaling, Abrahamsson *et al.* [47] found that spontaneous release in L5 PyCs was not sensitive to Mg^{2+} wash-out or MK-801 wash-in. This was furthermore in agreement with earlier findings that preNMDAR control of spontaneous release does not rely on Ca^{2+} [56]. Taken together, these findings suggest that preNMDARs depend on RIM1 $\alpha\beta$ and relief of Mg^{2+} blockade to control evoked release, but on JNK2 and metabotropic signaling to regulate spontaneous release [47] (**Fig. 3**). This interpretation helps to explain the long-standing issue of how preNMDAR-dependent regulation of evoked by not spontaneous release requires a critical frequency [3,5,10,21].

It is not clear, however, that the preNMDARs controlling evoked and spontaneous release are the same. In hippocampal cultures, for example, spontaneous and evoked release sites do not necessarily overlap [57]. A simple interpretation is that the same population of preNMDARs regulates both spontaneous and evoked release via two different paths (**Fig. 3**). Another possibility is that two preNMDAR populations are physically separated, e.g. by RIM nanocolumns [58]. A third possibility is that NMDARs modulate different bouton populations, one responsible for evoked release and the other for spontaneous release [57,59]. In any of these scenarios, the preNMDARs modulating evoked release would presumably be GluN1-GluN2B heterodimers, but the preNMDARs modulating spontaneous release could also be GluN1-N2B/N2C/N2D or GluN3-containing heterotrimers. These heterotrimers would explain the GluN2B pharmacological profile in combination with low Mg^{2+} sensitivity [18]. Finally, another possibility is that these vesicle pools are the same, but that the Ca^{2+} sensor for evoked and spontaneous release are distinct [60].

The schematic in **Fig. 3** is thus merely a parsimonious working model to illustrate the findings of Abrahamsson *et al.* [47].

Conclusions and future directions

For a long time, the preNMDAR field suffered from a lack of cell-type specific tools and powerful imaging techniques. However, it is currently well established that preNMDARs play key roles in different brain regions during both long-term and short-term plasticity [3-6]. Until recently, one outstanding question was how preNMDARs can modulate both evoked and spontaneous release when the former but not the latter is frequency dependent [3,5,10,21]. Abrahamsson *et al.* [47] addressed this question and found that preNMDARs employ specific and distinct pathways regulating evoked and spontaneous release separately.

This discovery, however, raises several other issues. Are these preNMDARs involved in evoked and spontaneous release the same? Do classical properties of postsynaptic NMDARs apply to preNMDARs? For example, preNMDARs have mixed pharmacological profiles, with reduced Ca^{2+} conductance, which may make them distinct from postsynaptic NMDARs [8,16,17,19]. PreNMDARs can apparently also signal metabotroically or ionotropically to control different aspects of synaptic release [47].

It is tempting to compare the preNMDAR debate with the controversy surrounding the pre- versus postsynaptic location of long-term synaptic plasticity, which was once quite heated [35-37]. Over the past two decades, however, this controversy has gradually given way to the realization that plasticity is determined by many different factors, such as animal age, induction protocol, and synapse type [35-37]. The controversy surrounding preNMDARs has echoed this debate. We believe the preNMDAR debate is going to be resolved in a similar manner: with

preNMDARs, like with LTP and LTD [61] , there is an embarrassment of riches. This diversity has ensured long-standing disagreement among neuroscientists. For this reason, we argue that elucidating unconventional NMDAR signaling — presynaptically as well as metabotroically — will help resolve the preNMDAR debate [28].

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Figure legends

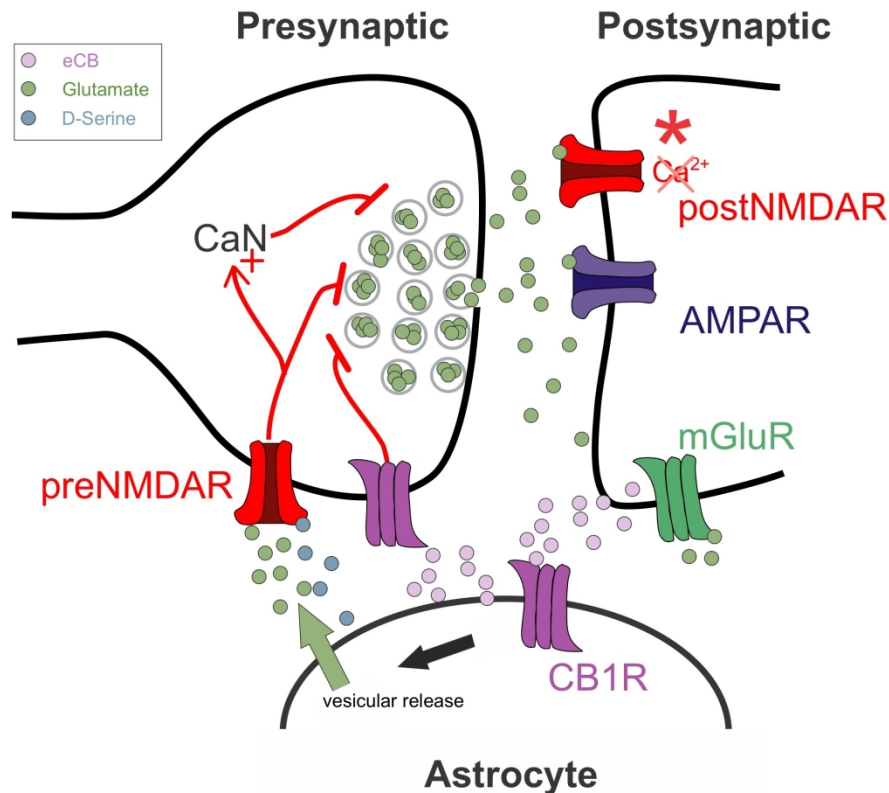


Figure 1 – Differing models of tLTD at L4 to L2/3 connections

In tLTD induction, presynaptic NMDARs and cannabinoid receptors (CB1Rs) are typically required for tLTD [although see 22]. CB1Rs are expressed both on L4 boutons and astrocytic processes, where they modulate the presynaptic release or elicit release of glutamate and D-serine, respectively. PreNMDAR-mediated Ca^{2+} activates calcineurin (CaN), which triggers a reduction in p_r . This model is based on many studies over two decades [8,10,19-25]. In contrast to these studies, Carter and Jahr [12] proposed that postsynaptic NMDARs (postNMDAR) but not preNMDARs trigger tLTD via metabotropic signaling (asterisk). They did not explore the expression mechanism [12].

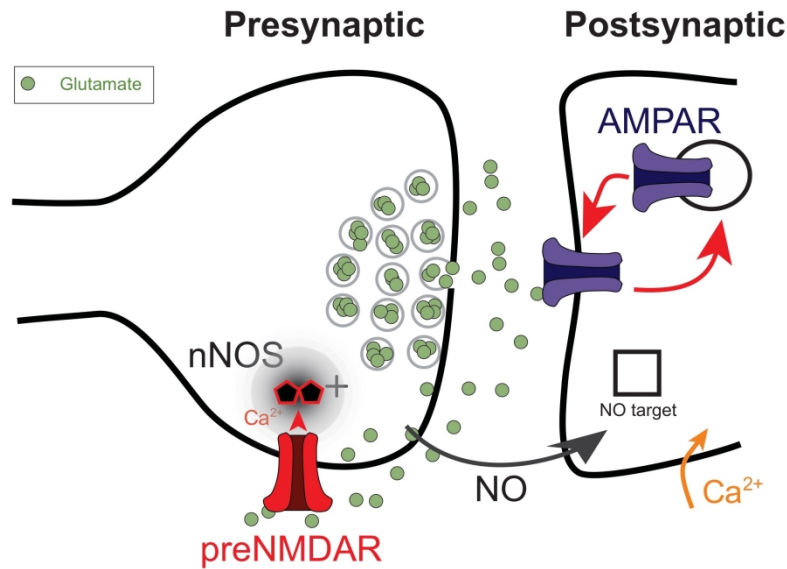


Figure 2 – Cerebellar long-term plasticity relies on preNMDARs and transsynaptic NO signaling

At PF-PuCs synapses, preNMDARs implement a bidirectional high-pass filter synaptic plasticity rule that is expressed postsynaptically [43,45]. High-frequency PF bursts activate preNMDARs, resulting in Ca^{2+} influx that activates neuronal nitric oxide synthase (nNOS), which is typically tightly coupled to NMDARs [62]. Nitric oxide (NO, black shadow) diffuses anterogradely to the postsynaptic side (black arrow). PF bursting produces NO in an NMDAR-dependent manner [44], while NO-mediated activation of guanylate cyclase is necessary for LTD induction [63,64]. In contrast, the targets of NO in LTP induction are unknown (black square: NO target), although guanylate cyclase is not required [42].

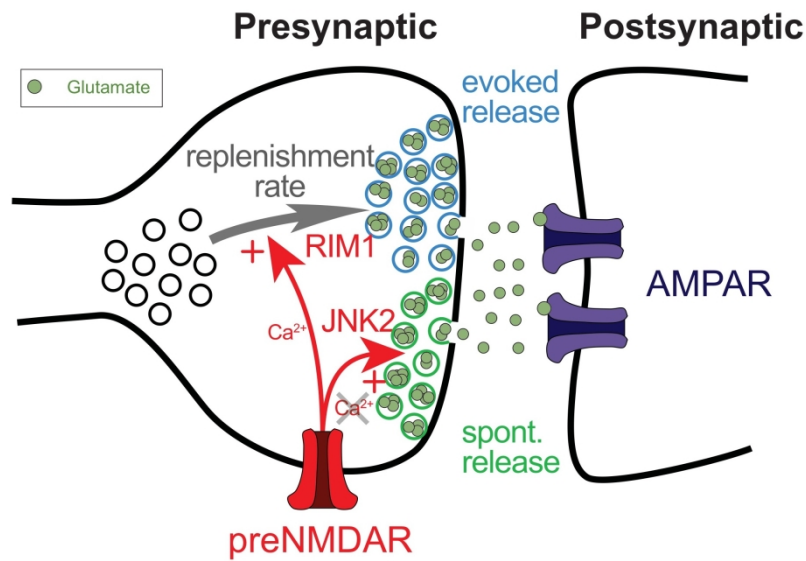


Figure 3 – At L5 excitatory connections, preNMDARs differentially control evoked and spontaneous release

Neocortical L5 PyC PreNMDARs rely on RIM1 $\alpha\beta$ and Ca²⁺ to upregulate the RRP replenishment rate, which sustains evoked release during periods of high-frequency firing. The upregulation of spontaneous release is separately controlled via JNK2 without Ca²⁺ or Mg²⁺ dependence [47,56]. This working model explains the different frequency dependencies of preNMDAR control of spontaneous and evoked release, and furthermore supports the view that spontaneous and evoked release are distinct processes [52].

Recommended reading articles

- of special interest
- of outstanding interest

•• Abrahamsson *et al.*, 2017 [47]

The authors find that neocortical preNMDAR regulation of evoked and spontaneous release is doubly dissociated: preNMDAR regulation of evoked relies on RIM1 $\alpha\beta$, whereas that of spontaneous depends on JNK2. To sustain rapid evoked release, preNMDARs boost p_r indirectly by upregulating RRP replenishment rate. Finally, spontaneous release regulation was not Mg²⁺ sensitive, suggesting metabotropic signaling.

•• Andrade-Talavera *et al.*, 2016 [17]

The authors investigated the requirements for induction of tLTP and tLTD at CA3-CA1 synapses in P12–18 mouse hippocampus. They found that tLTP requires postsynaptic NMDARs containing GluN2A and GluN2B subunits, while tLTD depends on non-postsynaptic NMDARs containing GluN2C or GluN2D subunits.

• Aow *et al.*, 2015 [31]

Using FRET between fluorescently tagged GluN1 subunit cytoplasmic domains and Protein Phosphatase 1 (PP1), Aow *et al.* obtain findings in hippocampal cultures consistent with a transient movement of PP1 by NMDAR ligand binding in the absence of Ca²⁺ flux. As PP1 drives AMPA receptor removal, this suggests metabotropic NMDAR signaling in LTD [also see 30].

•• Bouvier *et al.*, 2016 [43]

Using 2-photon Ca²⁺ imaging, cell-type specific deletion of preNMDARs, and computer modelling, the authors show that cerebellar PF-PuC preNMDARs underlie LTP. They show direct

evidence for Ca^{2+} entry via preNMDARs in a subpopulation of PF varicosities during high-frequency bursts.

- **Carter and Jahr, 2016 [12]**

Using 2-photon Ca^{2+} imaging, optogenetics, and slice electrophysiology at barrel cortex L4-L2/3 synapses, the authors found evidence that post- rather than presynaptic NMDARs underlie tLTD induction. They propose that postsynaptic NMDARs signal metabotroically, independent of Ca^{2+} .

- **Dore *et al.*, 2015 [30]**

Using FRET between fluorescently tagged GluN1 subunit cytoplasmic domains, Dore and colleagues show in hippocampal culture that glutamate binding can transmit conformational information into the cell in the absence of ion flow, consistent with metabotropic NMDAR signaling [also see 31].

- **Nistico *et al.*, 2015 [55]**

Using biochemical, morphological, and functional approaches, Nistico *et al.* find in entorhinal cortex that JNK and its scaffold protein JIP1 are expressed presynaptically. They also show that putative preNMDARs rely on JNK2 to modulate NMDA-evoked as well as spontaneous glutamate release, thus identifying a key preNMDAR signaling path.

- **Padamsey *et al.*, 2017 [39]**

The authors found that presynaptic CA3-CA1 LTD relied on preNMDARs activated by glutamate release. In contrast, presynaptic CA3-CA1 LTP did not require glutamate signaling. Presynaptic LTP depended on activation of postsynaptic L-type Ca^{2+} channels that trigger retrograde nitric-oxide signaling.

• Stein *et al.*, 2015 [33]

Spine elimination is widely thought to depend on NMDAR-mediated Ca^{2+} influx in conjunction with LTD. Combining 2-photon uncaging and time-lapse imaging with slice electrophysiology, Stein *et al.* suggest that in rat hippocampus, metabotropic NMDAR signaling drive spine shrinkage and LTD independent of Ca^{2+} .

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